REMARKS

The Invention

The invention features a biomaterial linked to a pharmaceutically active compound, typically by an amide or ester bond, and methods of formation and uses thereof.

Support for the Amendments

The specification has been amended to include priority information, information relating to the Sequence Listing, and to correct typographical errors. Claims 5, 10, 13, and 35 have been amended to correct typographical errors. No new matter has been added by any of these amendments.

Correspondence Address

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The Office Action

Claims 5-40 are pending. Claims 5-40 stand rejected for anticipation or obviousness in view of Harris et al. (U.S. Patent No. 5,932,462; hereafter "Harris").

Rejections under 35 U.S.C. § 102(e)

Claims 5-40 are rejected for anticipation by Harris. M.P.E.P. § 2131 states, "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." (citations omitted) Harris does not meet this standard.

The present claims are directed to pharmaceutical compositions and methods for their synthesis or use. Claim 5 (from which claims 6-12, and 17-21 depend) states:

> 5. A biomaterial formed from the cross-linking of two or more precursor components, wherein at least one of said precursor components has the formula:
>
> D-Y-C(O)-$(CH_2)_n$-S-$(CH_2)_2$-CO-X-P,
> D-Y-C(O)-$(CH_2)_n$-NH-$(CH_2)_2$-CO-X-P,
> D-Y-C(O)-$(CH_2)_n$-NH-U-P,
> D-Y-C(O)-$(CH_2)_n$-S-U-P,
> D-Y-C(O)-$(CH_2)_2$-S-L-S-CH_2-CH_2-CO-X-P,
> D-Y-C(O)-$(CH_2)_2$-S-L-S-U-P,
> D-Y-C(O)-$(CH_2)_2$-NH-L-S-CH_2-CH_2-CO-X-P,
> D-Y-C(O)-$(CH_2)_2$-NH-L-S-U-P,
> D-Y-C(O)-$(CH_2)_2$-S-L-NH-CH_2-CH_2-CO-X-P,
> D-Y-C(O)-$(CH_2)_2$-S-L-NH-U-P,
> D-Y-C(O)-$(CH_2)_2$-NH-L-NH-CH_2-CH_2-CO-X-P, or
> D-Y-C(O)-$(CH_2)_2$-NH-L-NH-U-P,
>
> wherein D is a pharmaceutically active moiety; n is 1 or 2; Y is O, NH, or N; L is a linear or branched linker; X is O or N; P is a water-soluble polymer or a water-swellable polymer comprising one or more conjugated unsaturated groups; and U is the product of the addition of a nucleophile to an electrophilic group that is attached to said polymer.

The claim is thus directed to a cross-linked composition in which one of the precursors has the following properties: (1) a water-soluble or water-swellable polymer having one or more conjugated unsaturated groups and (2) a secondary amino or thioether group

located α or β to an amide or ester bond to a pharmaceutically active moiety. This precursor is incorporated into the biomaterial by reaction with the conjugated unsaturated group(s) of the polymer moiety. Harris does not teach or suggest a composition having these properties.

Harris discloses a composition having the structure $poly_a$-P-CR(-Q-$poly_b$)-Z (col. 7, l. 67), where "[p]oly_a and $poly_b$ represent nonpeptidic and substantially nonreactive water soluble polymeric arms that may be the same or different" (col. 8, ll. 8-10), P and Q and linkers, R is a substantially nonreactive moiety, and Z contains a site that is either reactive towards a nucleophile or the product of a reaction with a nucleophile (col. 8, ll. 10-24). Since $poly_a$ and $poly_b$ of Harris are substantially nonreactive, these polymer moieties cannot contain reactive conjugated unsaturated groups and cannot be cross-linked as in the composition of claim 5. In fact, Harris explicitly states that with his composition "[c]ross linking is avoided." (col. 7, ll. 64-65)

Moreover, Harris does not teach or suggest the thioether or secondary amine structures of claim 5. While Harris does disclose amine and thioether linkages (col. 21 and col. 23), there are two differences between Harris and the instant compositions: (1) the linkages of Harris are branched unlike the linkages of claim 5, and (2) the linkages of Harris are directly to a polymer, where additional chemical groups are present in the compositions of claim 5. Thus, Harris teaches neither of the two common properties of the compositions of claim 5, and the rejection of claims 5-12 and 17-21 for anticipation should be withdrawn.

Claim 33 (from which claim 34 depends) recites:

> 33. A pharmaceutically active compound of the formula D-O_2C-$(CH_2)_n$-SH or D-N(O)C-$(CH_2)_n$-SH, wherein n is 1 or 2 and D is a pharmaceutically active moiety.

The compositions of claim 33, like those of claim 5, contain an unbranched secondary amino or thioether group located α or β to an amide or ester bond to a pharmaceutically active moiety. As stated above, Harris fails to disclose such structures. Furthermore, such structures cannot be inherent characteristics of the Harris compounds. The § 102 rejections of claims 33 and 34 should therefore be withdrawn.

Claims 13, 14, 22-27, and 35-39 are directed to methods of forming a biomaterial of the invention. Each of these methods, as stated in independent claims 13, 35, and 37, recites steps of (1) linking a pharmaceutically active compound to a polymer containing two or more conjugated unsaturated groups and (2) cross-linking the conjugated unsaturated groups. As stated above, Harris does not disclose or suggest polymers having unsaturated conjugated groups and explicitly avoids cross-linking. Thus, the rejection of claims 13, 14, 22-27, and 35-39 for anticipation may be withdrawn.

Claim 15 (from which claim 16 depends) states:

> 15. A method of treating or preventing disease, disorder, or infection in a mammal by administering to said mammal a biomaterial comprising a pharmaceutically active moiety, wherein said biomaterial has an ester or amide bond onto said pharmaceutically active moiety, said bond having a half-life of between 1 hour and 1 year in an aqueous solution at pH 7.4 and 37 °C.

This claim is directed to a method for the controlled release of a pharmaceutically active moiety from a biomaterial. As the instant specification states, "the presence of a thioether near the ester or amide bond that attaches the pharmaceutically active compound to the linker enhanced the rate of hydrolysis of the bond..." (pg. 89, ll. 18-21). The specification further states that amine groups may also increase hydrolysis of an ester or amide bond. Harris does not teach that a thioether or secondary amine linkage be used to couple to a pharmaceutically active moiety or that a composition having such a linker be administered to a mammal for the purpose of treating or preventing a disease, disorder, or infection. Since many other linkers are disclosed, and the linker may be attached to a non-pharmaceutically active moiety, e.g., a surface, Harris does not inherently teach the limitations of claim 15 either. The rejection of claims 15 and 16 for anticipation should be withdrawn.

Rejections under 35 U.S.C. § 103(a)

Claims 5-40 stand rejected for obviousness in view of Harris. M.P.E.P. § 2143 states:

> To establish a *prima facie* case of obviousness, ... there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings...[, and] the prior art reference... must teach or suggest all the claim limitations.

Applicants assert that this standard has not been met in the present case.

Regarding claims 5 and 33 (and their dependent claims), which are directed to compositions, as stated above, Harris does not teach or suggest all of the limitations of these claims. Claim 5 is directed to a cross-linked biomaterial. As stated above, Harris specifically excludes cross-linking between reactive groups on polymers and thus teaches away from the compositions of instant claim 5. In addition, claims 5 and 33 require an unbranched thioether or secondary amine α or β to an amide or ester bond onto a pharmaceutically active moiety, and this thioether or secondary amine is not directly coupled to a polymer. Harris does not disclose or suggest the unbranched structures recited in claims 5 and 33. Since Harris does not teach or suggest the limitations of claims 5 and 33, the rejection of claims 5-12, 7-21, and 33-34 for obviousness may be withdrawn.

Regarding claims 13, 14, 22-27, and 35-39, which are directed to methods of forming biomaterials, again, Harris does not teach or suggest the claimed methods. The cited claims are directed to linking a pharmaceutically active compound to a polymer having two or more conjugated unsaturated groups and then cross-linking the conjugated unsaturated groups. As Harris excludes cross-linking of polymers and employs polymers having only one reactive group, Harris does not teach or suggest the limitations of the claims directed to methods of forming biomaterials.

Claim 15 is directed to a method of treating or preventing a medical condition by administering a composition that contains an amide or ester bond that has a half-life of between 1 hour and 1 year. Harris does not teach or suggest such a method. Harris is silent with regard to a rate of hydrolysis. Thus, Harris fails to explicitly teach or suggest

the limitations of claim 15. Furthermore, although Harris discloses thioether and secondary amine linkers, Harris does not suggest (1) that these linkers be used to form hydrolytically unstable amide or ester bonds; (2) that these linkers be used to couple to a pharmaceutically active compound; or (3) that such a composition be administered to a mammal.

As the courts have held, to render a combination obvious, the prior art must do more than disclose the individual elements; the prior art must suggest the claimed combination as well.

For example, in the case of *In re Fromson*, 755 F.2d 1549, 225 U.S.P.Q. 26 (Fed. Cir. 1985), the claimed invention was a photographic plate for use in planographic printing. The district court held the patent invalid for obviousness, finding that "the Fromson patent is a combination patent comprised of old elements." The Federal Circuit, while conceding that the prior art disclosed all of the individual elements of the invention, reversed, stating:

> At no point did the court indicate, nor does the record indicate, a basis on which it can be said that the making of that combination would have been obvious when it was made.... The critical inquiry is whether "there is something in the prior art as a whole to suggest the desirability, and thus the obviousness, of making the combination." (emphasis in the original, citing *Lindeman Maschinefabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d at 1462, 221 U.S.P.Q. at 488.) Where, as here, nothing of record plainly indicates that it would have been obvious to combine previously separate process steps into one process, it is legal error to conclude that a claim to that process is invalid under § 103.

In the present case, as in *Fromson*, the cited art discloses some of the individual elements of the claimed methods. Harris teaches many different types of linkers other

than those containing a thioether or secondary amine (col. 19-23). In addition, while Harris discloses that their compositions may be used in prodrugs, they disclose myriad other uses and provide no specific teachings on the linkages between their polymeric composition and a drug. As to claims 15 and 16, Harris does not suggest the desirability of the unique combination of the claimed method, namely that a drug be coupled to a biomaterial via an amide or ester bond having a specified half-life. Nothing in Harris directs one skilled in the art to the particular types of amide or ester bonds of the claimed methods. Because Harris suggests a number of possible compositions and uses thereof, leading to a substantial number of possible specific combinations, some suggestion must also exist in the references for choosing and combining the particular elements of the present method from the wide variety of suggested approaches. No such suggestion is present. Accordingly, the § 103 rejection of claims 15 and 16 should be withdrawn.

New Claim 41

New claim 41 is directed to the method of claim 15, in which the biomaterial is cross-linked. As stated above, Harris does not teach or suggest the use of cross-linked materials, and thus new claim 41 is patentable over Harris.

CONCLUSIONS

Applicants submit that the claims are in condition for allowance, and such action is respectfully requested. Enclosed is a petition to extend the period for reply for three months, to and including August 6, 2002.

If there are any addition charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: August 6, 2002

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Version with Markings to Show Changes Made

A marked-up version of the paragraph starting on page 12, line 3 is as follows:

In a thirteenth aspect, the invention features a method of making a biomaterial. This method includes (a) attaching a pharmaceutically active compound to a linker molecule or incorporating a nucleophilic amine or thiol into a pharmaceutically active compound, (b) removing any thiol-or amine-protecting groups in the linker, (c) coupling a thiol, amine, or alkene group in the linker or incorporated into the pharmaceutically active compound to a water soluble polymer or a water swellable polymer having two or more conjugated unsaturated groups by a conjugate addition reaction to form a precursor component, and (d) cross-linking the uncoupled conjugated unsaturated groups in one or more of the precursor components. In one preferred embodiment, a polymer that has one or more conjugated unsaturated groups and that is not coupled to a pharmaceutically active moiety is incorporated into the biomaterial by performing the cross-linking in the presence of this polymer. In another preferred embodiment, the cross-linking is performed in the presence of a linker having two or more nucleophilic groups, and the linker is thereby incorporated into the biomaterial. Preferred linkers include a peptide with an amino acid sequence that is 80%, preferably 90%, or more preferably 100% identical to the sequence GCNNRGDNNCG (SEQ ID NO: 75[No. 73]). Other preferred linkers include those having an amino acid sequence or moiety that provides targeting to cells, tissues, organs, organ systems, or sites within a mammal. In one preferred embodiment, the cross-linking step and/or the formation of the precursor components of the biomaterial occurs within the body of a mammal, such as a human. In another preferred embodiment, the cross-linking occurs through free radical polymerization or conjugate addition reactions at or near a site within the body of a mammal. [Preferrably] Preferably, the cross-linking occurs through a self-selective reaction between a thiol or an amine and a conjugated unsaturated group. In another preferred embodiment, the cross-linking forms a hydrogel, a colloidal material, a microsphere, or nanosphere that can be delivered to a mammal, such as a human. In yet another preferred embodiment, the pharmaceutically active compound or a derivative thereof is released from the biomaterial and delivered to a site within the body. Preferably, the half-life the ester or amide bond onto the pharmaceutically active moiety is between 1 hour and 1 year at the site within the body. [Preferrably] Preferably, the half-life is between 1 hour and 1 year at pH 7.[ ]4 and 37 °C in an aqueous solution. The conjugated unsaturated groups of this aspect may have the same embodiments as listed for the conjugated unsaturated groups of any of the previous aspects.

A marked-up version of the paragraph starting on page 46, line 17 is as follows:

One can incorporate peptide sites for cell adhesion, namely peptides that bind to adhesion-promoting receptors on the surfaces of cells into the biomaterials of the present invention. It is straightforward to incorporate a variety of such adhesion-promoting peptides, such as the RGD sequence from fibronectin or the YIGSR (SEQ ID NO: 44) sequence from laminin. As above, this can be done, for example, simply by mixing a

cysteine-containing peptide with PEG diacrylate or triacrylate, PEG diacrylamide or triacrylamide or PEG diquinone or triquinone a few minutes before mixing with the remainder of the thiol-containing precursor component. During this first step, the adhesion-promoting peptide will become incorporated into one end of the PEG multiply functionalized with a conjugated unsaturation; when the remaining multithiol is added to the system, a cross-linked network will form. Thus, for example, when an adhesion peptide containing one cysteine is mixed with a PEG triacrylate (at, e.g., 0.1 mole of peptide per mole of acrylate end group), and then a protease substrate peptide containing two cysteine residues is added to form the three-dimensional network (at, e.g., equimolar less 0.1 mole peptide per mole of acrylate end group), the resulting material will be highly biomimetic: the combination of incorporated adhesion sites and protease sites permits a cell to establish traction in the material as it degrades a pathway for its migration, exactly as the cell would naturally do in the extracellular matrix *in vivo*. In this case, the adhesion site is pendantly incorporated into the material. One could also incorporate the adhesion site directly in to the backbone of the material. This could be done in more than one way. One way would be to include two or more thiols (e.g., cysteine) in the adhesion peptide or protein. One could alternatively synthesize the adhesion peptide (e.g., using solution phase chemistry) directly onto a polymer, such as PEG, and include at least one thiol (e.g., cysteine) or conjugated unsaturation per chain end.

A marked-up version of the table starting on page 72, line 1 is as follows:

Table 1. Plasmin Substrate Sites found in Fibrin (ogen) (Fg)**

Arginyl Sites								
P3	P2	P1	P1'	P2'	P3'	Fg chain and site	Reference	SEQ ID NO:
G	P	R+	V*	V*	E-	α 19	3	8
N	N	R+	D-	N	T	α 104	2, 4	9
Y	N	R+	V*	S	E-	α_110	2	10
Q	M*	R+	M*	E-	L*	α 239	1	11
G	F*	R+	H+	R+	H+	α 491	5	12
G	Y	R+	A*	R+	P	β 42	2, 3	13
Lysyl Sites								
Y	Q	K+	N	N	K+	α_78	3	14
L*	I*	K+	M*	K+	P	α 206	1, 2	15
N	F*	K+	S	Q	L*	α_219	1	16
E-	W	K+	A*	L*	T	α_230	1	17
S	Y	K+	M*	A*	D	α_583	5	18
T	Q	K+	K+	V*	E-	β_53	3	19
R+	Q	K+	Q	V*	K+	β_130	2	20
Q	V*	K+	D-	N	E-	β_133	4	21
L*	I*	K+	A*	I*	Q	γ_62	4	22
T	L*	K+	S	R+	K+	γ_85	2, 3	23
S	R+	K+	M*	L*	E-	γ_88	2	24

Ref. 1: Takagi T. and R.F. Doolittle, Biochemistry 14: 5149-5156 ,1975; Ref. 2: Hantgan R.R., et al., Hemostasis and Thrombosis: Basic Principles and Clinical Practice, Third Edition. Edited by R.W. Colman et al. J.B. Lippincott Company: Philadelphia, 1994; Ref. 3: Takagi T. and R.F. Doolittle, supra.; Ref. 4: Nomura S. et al., Electrophoresis 14: 1318-1321 1993.; Ref. 5: Ständker L. et al., Biochemical and Biophysical Research Communications 215: 896-902 (1995).

* Indicates a hydrophobic amino acid; +/- Indicates a charged side chain, either cationic (+) or anionic (-).

** Single letter amino acid code: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

A marked-up version of the table starting on page 73, line 4 is as follows:

Table 2. Collagenase Substrate Sites found in Collagen

P3	P2	P1	P1'	P2'	P3'	Collagen type and site	Ref.	SEQ ID NO:
P	Q	G	I*	A*	G	calf & chick α1(I); human cartilage α 1 (II)	6	25
P	Q	G	L*	L*	G	calf α 2 (I)	6	26
P	Q	G	I*	L*	G	chick α 2 (I)	6	27
P	Q	G	L*	A*	G	chick α 2 (I); human skin α 1 (III)	6	28
P	L*	G	I*	A*	G	human liver α 1 (III)	6	29
P	L*	G	L*	W	A*	human	7	30
P	L*	G	L*	A*	G	human	8	31

Ref. 6: Netzel-Arnett S. et al., The Journal of Biological Chemistry 266: 6747-6755 ,1991; Ref. 7: Upadhye S. and V.S. Ananthanarayanan, Biochemical and Biophysical Research Communications 215: 474-482 ,1995; Ref. 8: Liko Z., et al., Biochem Biophys Res Commun 227: 351-35 , 1996.

A marked-up version of the table starting on page 74, line 1 is as follows:

Table 3. Design of Collagenase (Matrix metalloproteinase I)-Sensitive Peptide Sequences

No.	Sequence	k_{cat}/ K_m relative to that of PQGIAG	SEQ ID NO:
1	GPQGIAGQ	100% (normal)	32
2	GPVGIAGQ	30% (slow)	33
3	GPQGVAGQ	9% (slower)	34
4	GPQGRAGQ	<5% (very slow)	35
5	GPQGIASQ	130% (fast)	36
6	GPQGIFGQ	>300% (faster)	37
7	GPQGIWGQ	>700% (very fast)	38

Netzel-Arnett S. et al., The Journal of Biological Chemistry 266: 6747-6755 ,1991

A marked-up version of the table starting on page 81, line 1 is as follows:

Table 4. Cell Binding Domain Sequences of Extracellular Matrix Proteins

Protein	Sequence	Role	SEQ ID NO:
Fibronectin	RGDS	Adhesion of most cells, via $\alpha_5\beta_1$	39
	LDV	Adhesion	N/A
	REDV	Adhesion	40
Vitronectin	RGDV	Adhesion of most cells, via $\alpha_v\beta_3$	41
Laminin A	LRGDN	Adhesion	42
	IKVAV	Neurite extension	43
Laminin B1	YIGSR	Adhesion of many cells, via 67 kD laminin receptor	44
	PDSGR	Adhesion	45
Laminin B2	RNIAEIIKDA	Neurite extension	46
Collagen I	RGDT	Adhesion of most cells	47
	DGEA	Adhesion of platelets, other cells	48
Thrombospondin	RGD	Adhesion of most cells	N/A
	VTXG	Adhesion of platelets	49

After Yamada, Y., and Kleinman, H.K., Curr. Opin. Cell Biol. 4:819, 1992.

A marked-up version of the table starting on page 82, line 1 is as follows:

Table 5. Proteoglycan Binding Domain Sequences of Extracellular Matrix Proteins

Protein	Sequence	SEQ ID NO:
χBBχBχ*	Consensus sequence	50
PRRARV	fibronectin	51
YEKPGSPPREVVPRPRPGV	fibronectin	52
RPSLAKKQRFRHRNRKGYRSQRGHSRGR	vitronectin	53
RIQNLLKITNLRIKFVK	laminin	54
K(βA)FAKLAARLYRKA	antithrombin III	55
KHKGRDVILKKDVR	neural cell adhesion molecule	56
YKKIIKKL	platelet factor 4	57

References for first five entries given in Massia, S.P., and Hubbell, J.A. J. Biol. Chem. 267:10133-10141, 1992; Antithrombin III sequence from Tyler-Cross, R., et al., Protein Sci. 3: 620-627, 1994; Neural cell adhesion molecule sequence from Kallapur, S.G., and Akeson, R.A., J. Neurosci. Res. 33: 538-548, 1992; Platelet factor 4 sequence from Zucker, M.B., and Katz, I.R., Proc. Soc. Exp. Biol. Med. 198, 693-702, 1991.

*χ indicates a hydrophobic amino acid. Basic amino acids are shown underlined.

A marked-up version of the paragraph starting on page 94, line 5 is as follows:

The subsequent cross-linking reaction can generally occur by two main routes, utilizing either free-radical polymerization (Lau *et al.*, *Bioorg. Med. Chem.* 3:1305-1312, 1995) or conjugate addition reactions. The conjugated unsaturated groups of the present invention that are reacted with good nucleophiles via conjugate addition reactions can generally also be polymerized by free-radical mechanisms. Thus, as long at least one conjugated [comjugated] unsaturated group remains on the polymer following the coupling of the pharmaceutically active compound, then that polymer can be incorporated into a biomaterial by free-radical mechanisms. The presence of at least one unreacted unsaturated group on the polymer is assured by keeping the number of unsaturated groups in excess compared to the thiol or amine groups present in, or coupled to, the pharmaceutically active moiety. The second route to cross-link these materials involves reacting the remaining conjugated unsaturated groups on the polymer coupled to a pharmaceutically active moiety with cross-linker molecules containing 2 or more nucleophiles, such as the peptide GCNNRGDNNCG (SEQ ID NO: 75[No. 73]) that increases cell adhesion to basement membranes. The cross-linking to form a material then occurs through another conjugate addition reaction.

A marked-up version of the paragraph starting on page 102, line 4 is as follows:

Gels were formed from PEG-2500-3A and GCYKNRDCG (SEQ ID NO: 58) as well as from PEG-3500-3A and GCYKNRDCG (SEQ ID NO: 58). Gels have been formed at three ratios of acrylates to sulfhydryls (1: 1, 1.1: 1, and 1.25: 1). Gels were formed in 10 mM phosphate buffered saline with triethanolamine to adjust the pH to 8.0-9.0 as tested by paper pH strips (gel formation reactions were performed at 50 microliter and smaller scales). Gels have been made by: predissolving the peptide and then adding peptide solution to PEG-3A; by predissolving the PEG-3A and adding its solution to the peptide; and by predissolving both solutions and then mixing them in appropriate ratios.

A marked-up version of the paragraph starting on page 102, line 18 is as follows:

2.5 mg of GCYKNRDCG (SEQ ID NO: 58) were weighed into an Eppendorf tube. 7.0 mg of PEG-2500-3A were weighed into a separate Eppendorf tube. 62 microliters of phosphate buffered saline (PBS)•TEA (10 mM PBS with 13 microliters of triethanolamine/ml) were added to the PEG-2500-3A to give a solution of 4.5 mg/40 microliters. The PEG solution was allowed to sit until the PEG-3A had dissolved (less than five minutes). 40 microliters of the PEG-3A solution were added to the peptide, which dissolved extremely rapidly. The pipet tip used for the transfer was used to stir the mixture for approximately 3 seconds. A 1 microliter sample was withdrawn to test the pH by a paper strip (pH range 1-11). The pH was approximately 8.0. After 20-30 minutes, a gel had formed.

A marked-up version of the paragraph starting on page 103, line 22 is as follows:

Gels were made with 0.1 g/ml, 0.15 g/ml, and 0.2 g/ml PEG-2500-3A at a 20 microliter scale. The gels contained 1.1 acrylates per sulfhydryl in the peptide (nucleophile) component, GCYKNRDCG (SEQ ID NO: 58). For gel formation, PBS buffers were adjusted to account for added acidity of additional peptide in higher concentration gels and to give reactions at pH 8.0-8.5. Gels were made in quadruplicate.

A marked-up version of the paragraph starting on page 106, line 3 is as follows:

13.9 mg PEG-2500-3A was dissolved in 69.5 microliters (5.0 mg/25 microliters) of PBS•TEA (10 mM PBS containing 13 microliters of triethanolamine/ml) containing GCGYGRGDSPG (SEQ ID NO: 61) at a concentration of 3.2 mg GCGYGRGDSPG (SEQ ID NO: 61)/ml. 7.0 mg GCYKNRDCG (SEQ ID NO: 58) was dissolved in 65 microliters of PBS•TEA (2.7 mg/25 microliter). The GCYKNRDCG (SEQ ID NO: 58) was filtered through a 0.22 micron filter. After 9 minutes of reaction time, the PEG-2500-3A/GCGYGRGDSPG (SEQ ID NO: 61) solution was separately filtered through a 0.22 micron filter. As soon as the filtrations were complete, equivolumes (25 microliters) of the two solutions were added to wells of a Corning flat-bottomed tissue culture treated polystyrene 96 well plates. As the second of the two precursor solutions was added, the pipet tip was used to stir the mixture for 2-3 seconds. Then the gels were allowed to set at 37°C.

A marked-up version of the paragraph starting on page 106, line 18 is as follows:

Conjugate addition gels were made with 0.1 g/ml PEG-2500-3A and 1.1 acrylates per sulfhydryl in GCYKNRDCG (SEQ ID NO: 58). The gels were swollen for 24 hours in Dulbecco's modified Eagle's medium (some in serum-free conditions and some in 10% fetal bovine serum) containing 1% antibiotic and antimycotic agents. Human foreskin fibroblasts (passage 7; passaged with trypsin/EDTA) were seeded onto the gels. From time points two hours to 48 hours, the cells remained round and did not spread. The cells became increasingly clumped together. The cellular behavior was independent of serum in the medium. Control cells seeded on tissue culture treated polystyrene spread normally.

A marked-up version of the paragraph starting on page 107, line 5 is as follows:

Conjugate addition gels were made with PEG-2500-3A, GCYKNRDCG (SEQ ID NO: 58), and an RGD-containing peptide (GCGYGRGDSPG (SEQ ID NO: 61)) incorporated in a pendant fashion. The gels were made with 0.1 g PEG-2500-3A/ml and 1.1 acrylates per sulfhydryl in GCYKNRDCG (SEQ ID NO: 58). The gels were swollen for more than 36 hours in Dulbecco's modified Eagle's medium (some in serum-free conditions and some in 10% fetal bovine serum) containing 1% antibiotic and antimycotic agents. When the RGD peptide was incorporated on one of every 12 acrylates of the PEG-2500-3A, human foreskin fibroblasts (passage 8; passaged by trypsin/EDTA) adhered to the gels (both those swollen in serum-free conditions and those in serum-containing medium). At 6 hours post-seeding, the cells were uniformly

distributed over the gel surface, and approximately 50% of the seeded cells were spread (in both medium conditions).

A marked-up version of the paragraph starting on page 111, line 3 is as follows:

Aqueous size exclusion chromatography was performed using a Shodex OHpak SB-803 column (Showa Denko, Tokyo, Japan), using UV detection, measuring absorbance from 200-400 nm. The eluent was phosphate buffered saline (10 mM sodium phosphate, 140 mM NaCl, pH 7.4). PEG diacrylate has maximum absorbance at 205 nm, whereas the peptide used, GCGYGRGDS (SEQ ID NO: 64) has absorbance maxima at 220 and 270 nm, due to the presence of amide bonds, and a tyrosine. PEG diacrylate was dissolved in 0.1 M phosphate buffer at pH 8 at a concentration of 25 μmol in 1 ml. A sample of the solution was separated using size exclusion chromatography, and the polyethylene glycol eluted as a single peak with an absorbance maximum at 205 nm, and no absorbance at 220 or 270 nm. Next, the peptide (12.5 μmol) was added to the PEG diacrylate solution, and reacted at room temperature for 5 min. A sample was then separated using size exclusion chromatography, and a single peak was detected, with absorbance maxima at 205, 220, and 270 nm, with the same retention time as PEG diacrylate. This indicated that the peptide reacted with the PEG diacrylate. Similar studies were performed using C18 chromatography, using a gradient from 95% water with 0.1% trifluoroacetic acid, 5% acetonitrile to 40% water with 0.1% trifluoroacetic acid, 60% acetonitrile. The peptide Ac-GCGYGRGDSP-NH_2 (SEQ ID NO: 62), eluted at about 20% acteonitrile, whereas PEG or PEG-3400 diacrylate eluted at about 40% acetonitrile. Incubation of 1 mol of the peptide per 2 mol of PEG-3400 diacrylate in buffered water at pH 8 led to the disappearance of the peptide-related peak that elutes at 20% acetonitrile, with the emergence of absorbance bands at 220 and 270 nm that coeluted with the PEG peak at 40% acetonitrile. Collecting the peaks and analyzing by MALDI-TOF mass spectrometry indicated that the PEG-associated peak contained a mixture of unmodified PEG-3400 diacrylate, and a new species with molecular weight that was the sum of the PEG-3400 diacrylate and the peptide molecular weights.

A marked-up version of the paragraph starting on page 113, line 1 is as follows:

The peptide Ac-GCGYGRGDSP-NH_2 (SEQ ID NO: 62) was dissolved in deionized water, and PEG-8000 diacrylate was dissolved in deionized water buffered with 10 mM HEPES and 115 mM triethanolamine at pH 8. After mixing 1 mol of the peptide per 2 mol of the PEG-8000 diacrylate, the reaction was followed by C18 chromatography, using a gradient from 95% water with 0.1% trifluoroacetic acid, 5% acetonitrile to 40% water with 0.1% trifluoroacetic acid, 60% acetonitrile. The peptide Ac-GCGYGRGDSP-NH_2 (SEQ ID NO: 62), eluted at about 20% acteonitrile, whereas PEG or PEG-8000 diacrylate eluted at about 40% acetonitrile. Rapidly, the free peptide peak at 20% acetonitrile disappeared, and the peptide then coeluted with the PEG peak at 40% acetonitrile. The solution containing the PEG-peptide adduct was then incubated at 37°C, and C18 chromatographic injections were made at later time points to detect hydrolysis of the peptide from the polymer. This was measured by observing the

decrease in signal at 273 nm that coeluted with the PEG peak, and the reappearance of the free peptide peak at about 20% acetonitrile. MALDI-TOF mass spectrometry of the new peak eluting at about 20% acetonitrile revealed a product of molecular weight which corresponded to the molecular weight of the original peptide plus 72 mass units. This indicated that the new peak contains peptide modified with propionic acid, which was the product that would be expected following conjugate addition between the cysteine on the peptide and an acrylate group, followed by hydrolysis of the ester of the modified acrylate. A half-life for hydrolysis of the ester between the peptide and the PEG was found to be 4.86 days. This corresponds to a half-life of hydrolysis of about 3 weeks at pH 7.4.

A marked-up version of the paragraph starting on page 114, line 16 is as follows:

13.9 mg PEG-2500-3A was dissolved in 69.5 microliters (5.0 mg/25 microliters) of PBS•TEA (10 mM PBS containing 13 microliters of triethanolamine/ml). 7.0 mg of GCYKNRDCG (SEQ ID NO: 58) was dissolved in 65 microliters of PBS•TEA (2.7 mg/25 microliter). Equivolumes (25 microliters) of the two solutions were added to wells of a Corning flat-bottomed tissue culture treated polystyrene 96 well plates. As the second of the two precursor solutions was added, the pipet tip was used to stir the mixture for 2-3 seconds. Then the gels were allowed to set at 37°C. The gels were then transferred to tubes containing 10 mM HEPES buffered saline, pH 7.4. The gels were incubated at 37°C, and the disappearance of the solid gels was followed visually. Between 14 and 21 days, all of the solid gels were gone, indicating that they had degraded by hydrolysis of the ester bond between the peptide and the PEG.

A marked-up version of the paragraph starting on page 115, line 5 is as follows:

13.9 mg PEG-2500-3A is dissolved in 69.5 microliters (5.0 mg/25 microliters) of PBS•TEA (10 mM PBS containing 13 microliters of triethanolamine/ml). 7.0 mg of GKKKKGCYKNRDCG (SEQ ID NO: 65) is dissolved in 65 microliters of PBS•TEA (2.7 mg/25 microliter). Equivolumes (25 microliters) of the two solutions are added to wells of a Corning flat-bottomed tissue culture treated polystyrene 96 well plates. As the second of the two precursor solutions is added, the pipet tip is used to stir the mixture for 2-3 seconds. Then the gels are allowed to set at 37°C. The gels are then transferred to tubes containing 10 mM HEPES buffered saline, pH 7.4. The gels are incubated at 37°C, and the disappearance of the solid gels is followed visually. The extra lysines found in the peptide ("GKKKK..." (SEQ ID NO: 73)) are added so as to provide additional nucleophiles to the local environment of the ester bond. Additionally, the cationic nature of the groups may also lead to a raising of the local pH. The combination of these two effects is expected to enhance the rate of hydrolysis of the ester bond between the peptide and the polymer.

A marked-up version of the paragraph starting on page 115, line 25 is as follows:

Since enzymes and peptides are chiral, the stereochemistry of GCYKNRDCG (SEQ ID NO: 58) was altered to make a plasmin-stable nucleophile for gels made by

conjugate addition. This plasmin stable peptide was: GCY-DLys-N-DArg-DCG (SEQ ID NO: 66). The sequence was otherwise not altered in order to maintain the extremely good water solubility properties of GCYKNRDCG (SEQ ID NO: 58).

A marked-up version of the paragraph starting on page 116, line 5 is as follows:

Analytical C18 HPLC (linear acetonitrile gradient over 0.1% TFA in water) was used to confirm the relative plasmin-stability of GCY-DLys-N-DArg-DCG (SEQ ID NO: 66). The following samples were run: plasmin; GCYKNRDCG (SEQ ID NO: 58); plasmin + GCYKNRDCG (SEQ ID NO: 58); GCY-DLys-N-DArg-DCG (SEQ ID NO: 66); and plasmin + GCY-DLys-N-DArg-DCG (SEQ ID NO: 66). Plasmin (micromolar) was present at 1/1000 the concentration of the peptide (millimolar) and hence did not affect overlaid [overlain] absorbance chromatograms. Overlaying the traces (absorbance at 220 nm or 278 nm) of the peptide elutions vs. those of the peptide + plasmin, demonstrated that [the] most of the GCYKNRDCG (SEQ ID NO: 58) peptide was degraded in approximately 1 hour at 37°C. The GCY-DLys-N-DArg-DCG (SEQ ID NO: 66) peptide however, was unaffected by the plasmin at 24 hours, and remained unaffected over the lifetime of the plasmin in the sample (sample injected for C18 at 2 weeks).

A marked-up version of the paragraph starting on page 116, line 20 is as follows:

Gels were made according to the 40 microliter protocol given above. Some contained the GCYKNRDCG (SEQ ID NO: 58) peptide with Lys and Arg in the L configuration. Another contained the GCY-DLys-N-DArg-DCG (SEQ ID NO: 66) instead. All were exposed to 0.2 units of plasmin in 200 microliters and incubated at 37°C. The L-Lys, L-Arg configuration of the peptide was readily degraded by the enzyme. In one case, after 6 hours no gel remained. The DLys, DArg configuration gel has not been shown to degrade by plasminolysis.

A marked-up version of the paragraph starting on page 117, line 19 is as follows:

PEG gels were prepared as described above, using the peptide GCGYGRGDSPG (SEQ ID NO: 61). Most cells have receptors that recognize the sequence GRGDSPG (SEQ ID NO: 74), and cells will interact with surfaces displaying immobilized RGD containing peptides. To test cellular interactions of cells with PEG gels containing peptides incorporated via conjugate addition, gels were formed and human umbilical vein endothelial cells were seeded onto the gels. The change in the shape of the cells on the surface was observed, which indicated that the cells were interacting with the peptides on the surface. The change in shape is referred to as spreading, and refers to the change of the cell shape from spherical to flattened and polygonal on the surface. No cell spreading occurred on the PEG gels without peptide, and the specificity of the GCGYGRGDSPG (SEQ ID NO: 61) peptide was confirmed by comparison with gels containing the peptide GCGYGRDGSPG (SEQ ID NO: 68), which contains the same amino acids, but in a different sequence, and which has no biological activity. Cells were seeded onto the gels at a concentration of 400 cells per mm^2, and the number of spread cells per area was

[were] counted at different times (see Figure 6). The experiments were performed using the normal cell culture medium. Cells could only spread on gels that contained the peptide GCGYGRGDSPG (SEQ ID NO: 61), which was incorporated into the gels utilizing a conjugate addition reaction.

A marked-up version of the paragraph starting on page 119, line 20 is as follows:

Microspheres are formed via conjugate addition cross-linking of PEG-triacrylate and the peptide GCYdKNdRDCG (SEQ ID NO: 66 [68]) as in Example 7, but additionally the peptide GCGYGRGDSPG (SEQ ID NO: 61) is also included in the reaction mixture, at a ratio of 1 GCGYGRGDSPG (SEQ ID NO: 61) to 8 GCYdKNdRDCG (SEQ ID NO: 66). The bioactive peptide is tested for the ability to localize microspheres to the surfaces of cells, as compared with microspheres containing no bioactive peptide.

A marked-up version of the paragraph starting on page 120, line 22 is as follows:

The protein myoglobin (17,000 Da) was released from hydrogels made by conjugate addition between thiols and acrylates. PEG-3500-3A at 0.2 g/ml in PBS, pH 7.4 was mixed with a solution of the plasmin sensitive peptide GCYKNRDCG (SEQ ID NO: 58) such that the concentration of thiols and acrylates was the same and the final concentration of PEG-3500-3A was 10% (precursor solution). To some of the precursor solution, myoglobin was added (5.2 µl of 9.8 mg/ml myoglobin solution per 195 µl of precursor solution). Myoglobin was chosen as a model protein for growth factors, such as BMP-2, because of its similar size. 200 µl aliquots of precursor solution with and without myoglobin were made onto hemostatic collagen sponges. To some control sponges 5.2 µl of the 9.8 mg/ml myoglobin solution were added without gel precursors. To some sponges, PBS was added instead of myoglobin. After gels had solidified within the sponges, each sample was incubated in 4 ml of 10 mM PBS, pH 7.4, containing 0.1% sodium azide to prevent bacterial and fungal contamination. At 6 hr, 12 hr, 24 hr, 2 d, 3 d, 7 d, and 13 d the solution phase was removed from each sample and replaced with fresh PBS with 0.1% sodium azide. After day 13, the solutions were replaced with 0.08 units of plasmin in 4 ml PBS, the discontinuity marked by the vertical line in Figure 7. Solutions were developed using the BIORAD/Bradford protein microassay and compared to a standard curve made from myoglobin solutions of known concentration. The samples with myoglobin within the hydrogel material showed a delayed release of the myoglobin (diffusion limited) but did, following hydrogel degradation by the enzyme plasmin, release a total amount of protein not different from the total released from the sponges alone (no hydrogel) (data not shown).

A marked-up version of the paragraph starting on page 125, line 3 is as follows:

For example, the DNA for vascular endothelial growth factor (VEGF) is modified using site directed mutagenesis to introduce a cysteine near the N terminus of the protein. Molecular biological techniques are used to synthesize, purify and fold the protein. The protein is incubated with PEG-triacrylate with acrylates in excess of thiols in the protein.

A plasmin sensitive peptide containing two thiols (GCYKNRDCG (SEQ ID NO: 58)) is added to cross-link the material with the growth factor incorporated throughout.

A marked-up version of the paragraph starting on page 127, line 9 is as follows:

Materials were made essentially according to Example 3, but under sterile conditions and with PEG-3500-3A, a molar ratio of acrylates: thiols of 1:1, and a molar ratio of GCGYGRGDSPG (SEQ ID NO: 61): acrylates of 1/12. At the time of gel formation, a recombinant human growth factor, BMP-2, which induces bone formation was added to the precursor solution at a concentration of 250 μg/ml of precursor solution. Precursor solution was added to hemostatic collagen sponges (Helistat; 8 mm diameter, approximately 3.5 mm height). Precursor solution was added until the sponges could not absorb more solution (approximately 160 μl). The gels were allowed to solidify in the sponges. Gels were briefly washed with PBS then kept minimally wet until implantation subcutaneously in rats. The implants were removed after two weeks, fixed, and hematoxylin and eosin stained. The materials were well infiltrated by cells with very little residual material remaining and promoted bone formation (mineralization and marrow formation) and vascularization. This indicates that the materials can deliver active biomolecules (e.g., growth factors) and can be infiltrated by cells *in vivo*.

Marked-up versions of claims 5, 10, 13, and 35 are as follows.

5. (Twice Amended) A biomaterial formed from the cross-linking of two or more precursor components, wherein at least one of said precursor components has the formula:

D-Y-C(O)-$(CH_2)_n$-S-$(CH_2)_2$-CO-X-P,
D-Y-C(O)-$(CH_2)_n$-NH-$(CH_2)_2$-CO-X-P,
D-Y-C(O)-$(CH_2)_n$-NH-U-P,
D-Y-C(O)-$(CH_2)_n$-S-U-P,
D-Y-C(O)-$(CH_2)_2$-S-L-S-CH_2-CH_2-CO-X-P,
D-Y-C(O)-$(CH_2)_2$-S-L-S-U-P,
D-Y-C(O)-$(CH_2)_2$-NH-L-S-CH_2-CH_2-CO-X-P,
D-Y-C(O)-$(CH_2)_2$-NH-L-S-U-P,
D-Y-C(O)-$(CH_2)_2$-S-L-NH-CH_2-CH_2-CO-X-P,
D-Y-C(O)-$(CH_2)_2$-S-L-NH-U-P,
D-Y-C(O)-$(CH_2)_2$-NH-L-NH-CH_2-CH_2-CO-X-P, or
D-Y-C(O)-$(CH_2)_2$-NH-L-NH-U-P,

wherein D is a pharmaceutically active moiety; n is 1 or 2; Y is O, NH, or N; L is a linear or branched linker; X is O or N; P is a water-soluble polymer or a water-swellable polymer comprising one or more conjugated unsaturated groups; and U is the product of the addition of a nucleophile to an electrophilic group that is attached to said polymer.

10. (Amended) The biomaterial of claim 5, wherein said conjugated unsaturated groups are selected from the group consisting of acrylates, methacrylates, acrylamides, methacrylamides, acrylonit[i]riles, and quinones.

13. (Amended) A method of forming a biomaterial, said method comprising the steps of:

(a) attaching a pharmaceutically active compound to a linker molecule or incorporating a nucleophilic amine or thiol into a pharmaceutically active compound,

(b) removing any thiol- or amine-protecting groups in said linker,

(c) coupling a thiol, amine, or alkene group in said linker or incorporated into said pharmaceutically active compound to a water-soluble polymer or a water-swellable polymer comprising two or more conjugated unsaturated groups by a conjugate addition reaction to form a precursor component, and

(d) cross-linking the uncoupled conjugated unsaturated groups in one or more of said precursor components.

35. (Amended) A method of forming a biomaterial, said method comprising the steps of:

(a) attaching a pharmaceutically active compound to a linker molecule or incorporating a nucleophilic amine or thiol into a pharmaceutically active compound;

(b) coupling the thiol[,] or amine in said linker or incorporated into said pharmaceutically active compound to a polymer comprising two or more conjugated unsaturated groups by a conjugate addition reaction to form a precursor component; and

(c) cross-linking the uncoupled conjugated unsaturated groups in one or more said precursor components.